

GROWTH AND REPRODUCTION OF THE GIANT GLOSSIPHONIID LEECH *HAEMENTERIA GHILIANII*

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ABSTRACT

A continuously breeding laboratory colony of the giant leech *Haementeria ghilianii* has been established from a few specimens collected in French Guyana. The leeches feed on live rabbits or bovine blood, which they draw from an artificial feeding device. Leech growth is saltatory, in that at each of four successive feedings, spaced over about half a year, the weight of each specimen increases 3- to 6-fold. The male reproductive system of this hermaphroditic leech matures first, at a body weight of 3–5 g. The female reproductive system matures after at least one more feeding, at a body weight of at least 8 g. Fecundity (number of eggs per laying) depends on the weight of the leech at the time of oviposition, increasing from 60 for the smallest mature individuals to 500 for the largest. These data on the growth and reproduction of *H. ghilianii* gathered under laboratory conditions are consistent with its probable life cycle in the native habitat.

INTRODUCTION

In the latter part of the 19th century, C. O. Whitman, the first director of the Woods Hole Marine Biological Laboratory, pioneered the use of glossiphoniid leeches for the study of animal development (Whitman, 1878, 1887). Using such leeches, Whitman was the first to show that a definite developmental fate can be assigned to the descendants of identifiable cells of an early embryo. By the turn of the century, leeches had become objects of intense interest, not only to embryologists but also to the founders of modern neuroanatomy, who, in their efforts to correlate the structure of the nervous system with its function (Leydig, 1862; Retzius, 1891), were attracted to the relatively simple, readily accessible, and highly stereotyped leech nervous system.

Unaccountably, interest in leeches as materials for embryological and neurological studies declined in the first decades of this century. Neurological interest in leeches was revived only in the 1960s, when it was found that modern electrophysiological methods, such as the recording of nerve cell activity by means of intracellular electrodes, can be applied with great profit to the leech nervous system (Nicholls and Kuffler, 1964). Meanwhile, such neurobiological studies have provided far-reaching insights into the leech nervous system, particularly its identified nerve cells and their synaptic connections (Nicholls and Van Essen, 1974). Moreover, these studies have led to a neurological account of moderately complex behavioral routines, such as the leech swimming movement, in terms of networks of identified neurons (Stent *et al.*, 1978). In view of these recent advances, the time seemed favorable to resume the embryological investigation of leeches as well, particularly within the context of contemporary developmental neurobiology.

Received 14 October 1980, accepted 14 January 1981.

However, the European medicinal leech, *Hirudo medicinalis*, with which most of the recent neurobiological work has been carried out, is not a particularly favorable species for embryological studies. *H. medicinalis* belongs to the Hirudinidae, whose members lay very small eggs and are sustained by larval organs in their early development. However, the members of another family, the Glossiphoniidae (studied by Whitman), lay larger eggs and are sustained by their yolk (Schleip, 1936). Thus the glossiphoniid egg is a more favorable object for the study of leech development.

For this reason we sought a glossiphoniid species with the following desirable properties: (1) easy and continuous breeding under laboratory conditions; (2) large eggs amenable to embryological analysis and manipulation; and (3) a nervous system amenable to neurophysiological analysis. Adults of most members of the Glossiphoniidae are so small that they lack the third property. But in 1849, one specimen of an exceptionally large glossiphoniid, designated *Haementeria ghilianii*, was discovered near the mouth of the Amazon (Filippi, 1849a, b). The species' existence subsequently was confirmed both in Brazil (Lang, 1890) and in neighboring French Guyana (Blanchard, 1899). Adult specimens were reported to reach lengths of up to 50 cm in full extension. Hence it appeared that this giant tropical glossiphoniid, of which no report had been made since 1899, might possess all three of the desirable properties. Accordingly, we secured live specimens of *H. ghilianii* in French Guyana. With these specimens we established a continuously breeding colony in 1976 at the University of California at Berkeley (Sawyer, 1978). Studies carried out on embryos and adult specimens obtained from that colony have shown that *H. ghilianii* is indeed amenable to developmental and neurophysiological analysis.

This article reports observations on the feeding habits, dynamics of growth, and development of reproductive potential of *H. ghilianii* under laboratory conditions.

MATERIALS AND METHODS

The *H. ghilianii* founder specimens of the present laboratory colony were collected in a marsh near Sinnamary, French Guyana. The water of the native marsh of these specimens is acidic (pH 5.8–6.2), undergoes diurnal temperature variation from about 25°C–35°C, and has a low oxygen content, particularly at the bottom. Being near the equator, the marsh has about 12 h of daylight throughout the year.

To approximate these conditions in the laboratory, the leeches are maintained in shallow, 2.6 m × 1.2 m basins filled to a depth of about 5 cm with deionized water to which is added 0.63 mM NaCl, 0.07 mM CaCl₂, 0.07 mM MgCl₂, and 0.01 mM K₂SO₄, to provide cations in their approximate natural concentrations. Buffering at pH 5.9 is provided by adding 1 mM morpholine ethane sulfonic acid (MES). The water is filtered or changed periodically to prevent waste products, such as ammonia from leech urine, from rising to toxic levels. After feeding, it is essential to change the water daily for several weeks. The water temperature is 25°C–27°C. The basins are in a greenhouse where the midday natural light intensity (measured at the water surface) varies from about 150 lux (on a cloudy day) to more than 500 lux (on a sunny day). In addition, incandescent light at an intensity of 100 lux at the water surface is provided year-round from 0600 to 1800 h.

Under our laboratory conditions *H. ghilianii* has been found to feed on the blood of mammals (cattle, rabbit, rat, mouse), less readily on reptiles (turtles), and not at all on fish (goldfish) or amphibia (frog). To withdraw blood from its mam-

malian or reptilian host, the leech attaches itself to the host's skin by means of its anterior sucker, and inserts its proboscis hypodermically. For economical laboratory feeding of adult *H. ghilianii*, we devised an artificial blood feeding device: This is a 23-cm-long plexiglass box with two compartments, each 10 cm in width, separated by a freshly prepared piece of skin from a cow's head. The compartment faced by the inner surface of the cow skin is filled to a depth of about 10 cm with fresh, heparinized (6 mg/l heparin salt) bovine blood. The other compartment, faced by the outer, hairy surface of the skin, is filled to the same depth with water. Hungry leeches placed in the water compartment attach themselves to the piece of skin, pierce it, and take in blood. Upon withdrawal of the proboscis after feeding, the small hole pierced in the skin by the proboscis closes, so there is little leakage between the two compartments.

The artificial feeding device is not used for the first blood meal of juvenile leeches, since a juvenile could find the cow skin too thick for its smaller proboscis. Hence, for the first feeding, the brooding parent leech, to whose venter the juveniles are still attached, is placed in an aquarium with an adult rabbit, whose ventral fur has been shaved. The aquarium is filled with enough water to cover the rabbit's venter. Under these conditions, juveniles ready for their first meal leave the parent, attach themselves to the rabbit, and draw its blood while they feed, later returning to the parent.

RESULTS

Reproduction

H. ghilianii, like all leeches, is a protandrous hermaphrodite. To mate, the sperm donor leech brings its male gonopore into contact with the body of another leech and ejaculates one spermatophore, which becomes implanted in the body wall of the recipient. Fertilized eggs issue from the female pore enclosed in a membranous sac, or cocoon, which remains attached to the ventral body wall of the brooding parent. Embryonic development of the fertilized egg begins upon oviposition and proceeds directly, via a series of highly stereotyped cleavages, to the formation of a juvenile leech attached by its posterior sucker to the venter of the brooding parent (Fig. 1). The embryonic development of *H. ghilianii*, which will be described in detail elsewhere, generally is similar to that previously reported for other glossiphoniid leeches (Schleip, 1936), especially for *Helobdella triserialis* (Weisblat *et al.*, 1978, 1980) and *Theromyzon rude* (Fernandez, 1980; Fernandez and Stent, 1980). The egg of *H. ghilianii* is 2.0–2.5 mm in diameter, of density 1.15 gm/cm³, and weighs 5–8 mg. It is rich in yolk, which sustains the embryo until, as a juvenile, it has become capable of feeding. Individual *H. ghilianii* juveniles shortly before feeding weigh 10–18 mg, or about twice as much as the eggs from which they developed. Most of that increase in weight must come from water assimilated into body tissues during embryogenesis.

Dynamics of growth

To ascertain when the leeches are ready to take their first meal, *i.e.*, reach the status of juveniles, a cohort of 366 hatchlings brooded by a single parent was given the opportunity to feed on a rabbit about once a week. Of the cohort 19% took their first meal on the 27th day after oviposition and 67% on the 33rd day. All but four of the remaining juveniles took their first meal on the 40th day.

To ascertain the trophic effects of feedings, the growth of eight juveniles was



FIGURE 1. (Left) *H. ghilianii* juveniles attached to the venter of their parent. The length of this specimen is about 10 cm from head (top) to the circular caudal sucker (bottom). (Photograph courtesy of Timothy Branning.)

FIGURE 2. (Right) *H. ghilianii* juveniles just before (right) and just after (center and left) their first feeding. Bar: 1 cm.

followed through sexual maturity. At first feeding, of about 20 min, the juveniles ingested about 6 times their pre-feeding body weight in blood (Fig. 2). The animals would not feed again for about a month, presumably until the ingested blood had been metabolized. The long-term increase in body weight resulting from the first feeding approximately equaled the amount of blood ingested. About a third of the weight of ingested blood was eliminated as urine within a first few days after feeding (*cf.* Table I), some fraction of the first meal probably was released as CO_2 and feces, and the remainder of the long-term weight increase must have reflected assimilation of water into the body tissues.

At their second feeding (Table I) of about an hour, the leeches ingested about 5 times their body weight in blood. During the ensuing 2 months, digestion and assimilation of the second meal again resulted in a long-term increase in weight about equal to that of the ingested blood. At the third feeding, the blood intake was again about 5 times the body weight, resulting in an approximately equal increase in long-term body weight during the following 3 months. Finally, at the fourth feeding, which lasted nearly 2 h, the blood intake was about 3 times the pre-feeding weight. Digestion of this fourth meal required 3 months or more.

Thus, as shown in Figure 3, the post-embryonic growth of *H. ghilianii* is best described as saltatory. The relative size of each step remained roughly constant but the duration increased progressively. However, among individuals of the same cohort the parameters of this saltatory development varied considerably—partic-

TABLE I

Weight changes associated with successive feedings of H. ghilianii. Data are mean values of measurements made on 8 isolated specimens of H. ghilianii whose individual growth was monitored from juvenile to sexually mature adult. All four feedings were on a rabbit host.

Number of Feeding	First	Second	Third	Fourth
Days since first feeding	0	36	92	180
Feeding time (minutes)	22	56	—	110
Body weight before feeding (grams)	0.017	0.11	0.74	4.6
Blood ingested (grams)	0.093	0.54	3.4	14.2
Relative increase in body weight during feeding	640%	580%	560%	400%
Ingested blood retained after urine elimination (grams)	0.063	0.44	2.7	11.8
Long-term increase in weight of body tissues due to feeding (grams)	0.096	0.63	3.9	11.4

ularly the amount of blood ingested per feeding, and hence the increase in body weight. In the eight leeches followed individually for securing the data of Table I, this variation resulted in a distribution of individual weights after the fourth feeding so broad that its standard deviation was about half the mean.

Under our laboratory conditions, the leeches live 1 to 2½ years: Of mature individuals (including the eight specimens reported in Table I and Figure 3), five died between the ages of 12 and 18 months, two died between the ages of 18 and 24 months, and the remaining four died between the ages of 24 and 29 months.

Maturation

The development and maturation of the male and female reproductive systems of *H. ghilianii* was related to body weight, and hence to the number of meals the animal had taken. The male reproductive system matured first at body weights of about 3–5 g. (Spermatophore discharge was not observed from individuals of lesser weight, whereas such discharges were common in the 3–5 g weight range.) By contrast, the female reproductive system did not normally mature until at least one more feeding, when body weight exceeded 10 g (Fig. 6). Some individuals laid eggs after only three feedings, but these already weighed 8–16 g.

Some time intervenes between the feeding that enables the female reproductive system to mature, and egg-laying. During that time the ingested blood is presumably digested and assimilated. Thus, of 129 individuals that became gravid during 1 year, none had fed within 2 months, 25% had fed within 3 months, 75% had fed within 5 months, and all had fed within 7 months. The time elapsed between feeding and oviposition appears to depend on such variables as the age and physiological state of the individual at the time of feeding and its weight after feeding. As shown in Table II, even among a cohort of six leeches, the time between the fourth feeding and egg-laying ranged from 2–5 months. Some leeches lay a second clutch of eggs as early as 2 months after the first clutch, without an intervening feeding or mating.

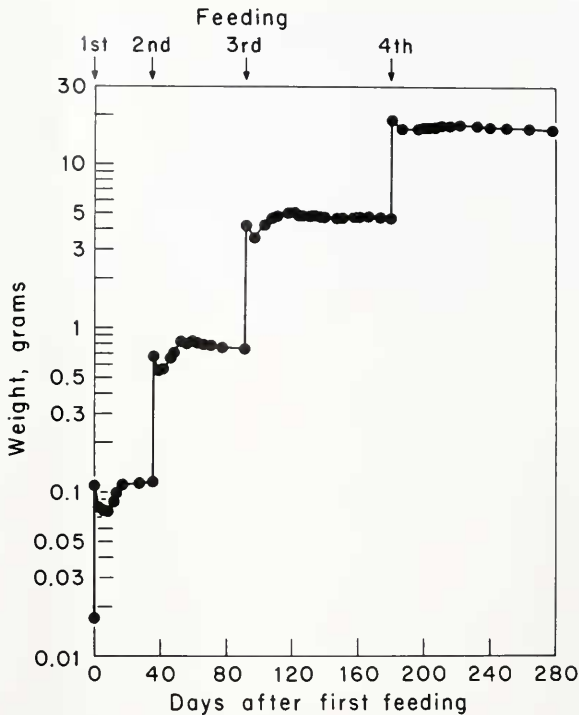


FIGURE 3. Growth dynamics of *H. ghilianii*, from juvenile to sexually mature adult, from mean weight of a cohort of eight isolated specimens provided four feedings on a rabbit host. The loss of weight in the first few days after feeding is presumably attributable to the elimination of fluid as urine.

After the female reproductive system matures, the male system retains its capacity to implant spermatophores. To ascertain where the donor leech implants its spermatophores on the recipient, we surveyed the location of spermatophores on the bodies of reproductively mature specimens. This survey showed (Fig. 4) that donors implant spermatophores onto all parts of the recipient's dorsal and ventral body surface except the head, and most commonly on the dorsolateral surface of

TABLE II

*Time between feeding and egg-laying: Six *H. ghilianii* specimens belonging to a cohort that had its third feeding 8 months earlier were given their fourth feeding on the same rabbit. Individual leeches laid eggs after the number of days shown had elapsed. The eggs were removed and counted, and the leech was then weighed.*

Leech Number	Days Between Feeding and Egg-Laying	Number of Eggs Laid	Weight of Leech (g)
1	58	149	16.0
2	59	275	24.8
3	65	272	23.5
4	75	232	22.3
5	108	246	21.5
6	152	321	30.5

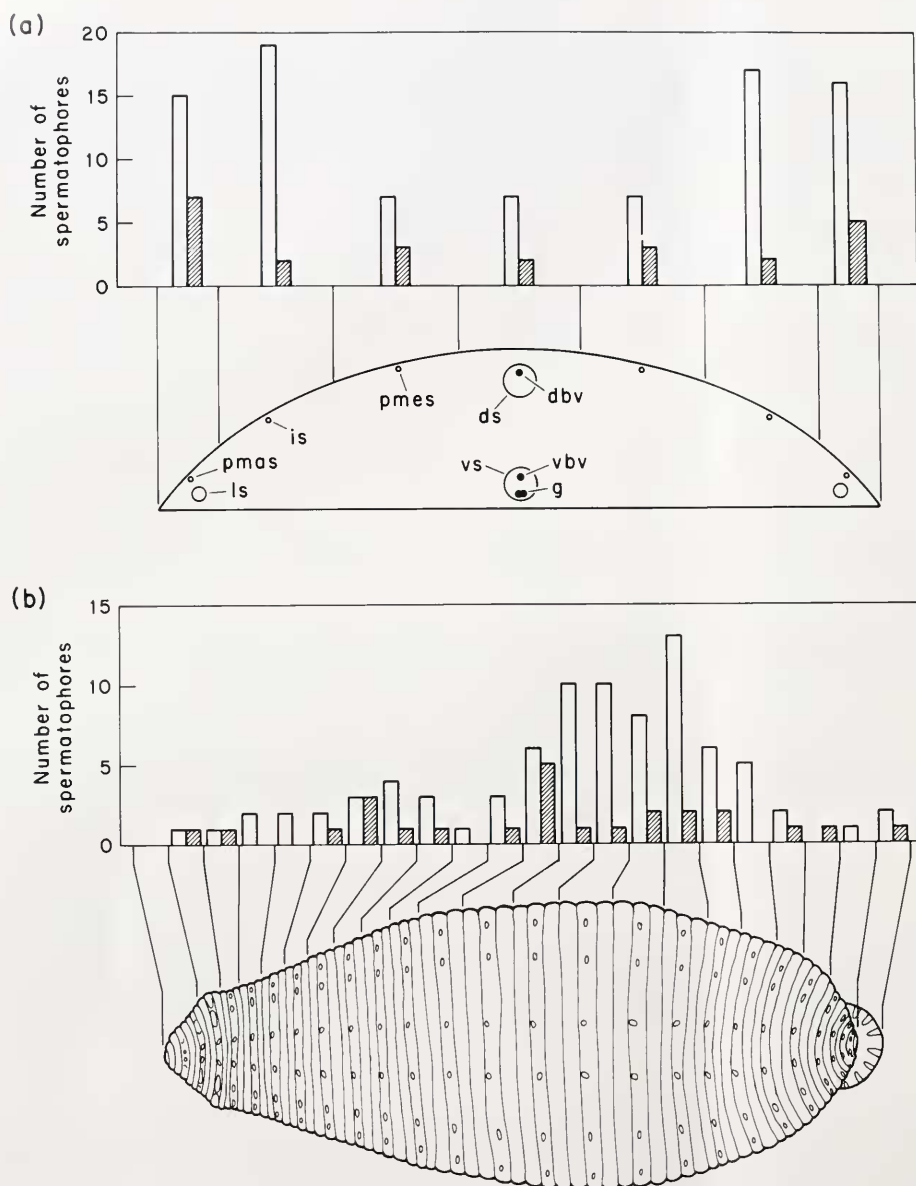


FIGURE 4. Distribution of implanted spermatophores over the body surface of a population of *H. ghilianii* adults. Open or crosshatched bars indicate the number of spermatophores found on the dorsal or ventral surface, respectively, within the domain delimited by the lines drawn to the body plan shown below the histogram. (a) Mediolateral distribution over 7 arbitrary sectors of the transverse cross-section. dbv = dorsal blood vessel; ds = dorsal sinus; g = ganglion; is = intermediate sinus; ls = lateral sinus; pmas = paramarginal sinus; pmes = paramedial sinus; vbv = ventral blood vessel; vs = ventral sinus. (b) Rostrocaudal distribution over the body segments shown at the bottom. Open circles represent sensillae marking the central annulus of each body segment.

the posterior body segments. This concurs with Myers' (1935) survey of spermatophore-implantation sites on *H. parasitica*. These preferred sites of implantation are close to the sinuses of those segments in which the eggs are carried

(Mann, 1962), thus minimizing the distance that the sperm released from the implanted spermatophore have to migrate to reach the eggs.

Gravid leeches could be recognized some weeks before they laid eggs, by a whitish clitellum that appeared in the ventral region surrounding the gonopores. Closer to the time of egg-laying, a large yellow area, showing yolky eggs in the ovisacs, could be seen on the ventral midline posterior to the gonopores. About 2 weeks before leeches laid eggs, the ventral inflection of the lateral margins of the body gradually increased. This inflection eventually produced a brood pouch that protected the eggs in their cocoons attached to the venter of the parent (Fig. 5). To ensure fertilization of their eggs under the conditions of laboratory culture, gravid individuals were placed in a separate container with a consort of about 6 active sperm-donor individuals.

The cocoons were normally deposited in pairs, one on each side of the ventral midline. When the number of cocoons formed was uneven, one unpaired cocoon usually took a medial position at the front. Figure 6, the results of a study of the fecundity of 125 *H. ghilianii* individuals, shows that the number of eggs per individual per laying increased with body weight, from about 100 for the smallest leeches with mature female reproductive systems (10 g) to about 350 for the largest leeches (50 g). (One exceptionally large, 80 g leech laid nearly 500 eggs.) Table II also shows that the number of eggs laid increased with body weight. In contrast, the number of cocoons per individual showed no significant relation to body weight; the number varied between 6 and 12, with a typical value of 10. Thus, the increase with maternal weight in egg number per individual was accompanied by an increase in mean number of eggs per cocoon per individual, from a low of about 10 for the 10 g individuals to a high of about 35 for the 50 g individuals.



FIGURE 5. *H. ghilianii* brood pouch, in which cocoons (with their eggs and early embryos) are held.

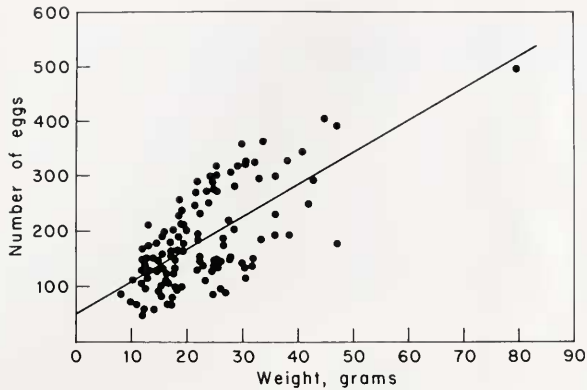


FIGURE 6. Fecundity of leeches as a function of body weight in terms of the number of eggs laid per individual. Each point represents the oviposition of one leech of weight shown on the abscissa. The straight line is a least-squares fit to the data points.

DISCUSSION

The data presented here on the laboratory rearing of *H. ghilianii* provide some insights into the life cycle of the leech in its native French Guyana habitat. In accord with the saltatory post-embryonic growth dynamics of laboratory-reared *H. ghilianii* (Fig. 3), the natural population in the marsh where the colony originated consisted of apparent weight classes, corresponding to laboratory-reared individuals after one, or two, or more feedings (Sawyer and LePont, unpublished observations). Similarly, Herter (1936) reported that natural populations of the glossiphoniid leech *Theromyzon tessulatum* comprise specimens falling into four distinct size classes, and inferred from this that leeches' mode of growth is "fractionated." One critical ecological factor in the *H. ghilianii* life-cycle is the dry season, from September to November. During that season the marsh's water level falls and daytime water temperature rises (up to 34°C at the surface). The leeches avoid these adverse conditions by remaining burrowed in the bottom mud, surviving by metabolizing previously ingested blood. Upon return of the rains in December, presumably much of the population is hungry; leeches emerge from the bottom mud to take another meal (Sawyer and LePont, unpublished observations). Thus we can expect that many reproductively mature individuals will feed synchronously after the dry season. Since the time of egg-laying is critically dependent on when the leeches feed, we predict that a wave of egg-laying occurs in the population after the dry season. On the basis of our laboratory data (Table II), this egg-laying wave would occur 2–7 months after the dry season ends, or normally in February to July.

ACKNOWLEDGMENTS

We thank Ian Madin for innovative contributions to this project, Milan Tomic, Brian Jennison, and Mooyung Choi for maintenance of the leech colony, and Margery Hoogs and Gunther Stent for helpful discussions and suggestions for the preparation of the manuscript. This work was supported by NIH Research Grant HL 22227; NIH Postdoctoral Fellowship NS 05996; NSF Postdoctoral Fellowship SPI-79L4849; a Postdoctoral Fellowship from the Miller Institute for Basic Research in Science; and a grant from the Penrose Fund of the American Philosophical Society.

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